Progress Report (July 1, 2021- April 1, 2022) **Agreement** #21-0426-000-SA

Submitting Organization: University of California Riverside

Project Title: Development and validation of virulence markers for vineyard phylloxera

Project Period: Year:1

Amount Requested: $ 33,014

Principal Investigators:

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Objectives

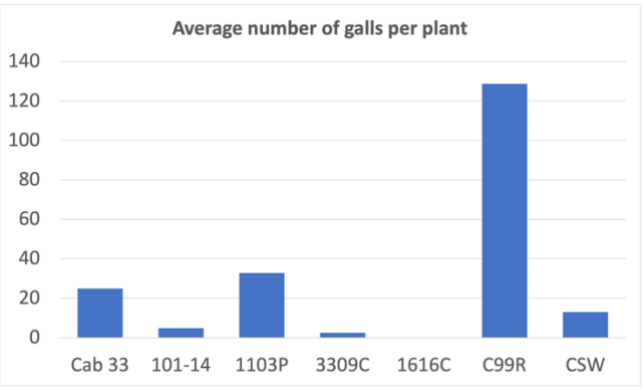
1. Determine the performance-based gene expression of phylloxera clones across rootstocks.

Summary: We established a clonal colony of grape phylloxera from St George rootstocks from the USDA-ARS grape repository near Davis, CA. Preliminary screens for leaf colonization were performed in a greenhouse in August 2021. These indicated this clone successfully colonized all *Vitis vinifera* genotypes but failed to colonize several native North American species and two rootstocks planned for use in this trial. Physical challenges in the greenhouse (neighbors misuse of pesticides and suboptimal climate control because of use of shared space) and slowing plant vigor because of the approaching fall season ended this screen prematurely. However, these data allowed us to characterize a signature of local adaptation or preference in the clone for genotypes with similar parentage (Figure 1).

To refine methodology, a full trial was established on roots in growth chambers in fall 2021. Necrosis after 1 month at feeding sites reduced the stability of the performance assay and suggested the leaf trial produced more reliable data on true resistance responses. Thus, a fully replicated leaf performance experiment was planned for Spring 2022 and is currently underway (Figure 2).

Modifications: Trials of chamber-grown rootstocks with root enclosure vessels designed to monitor insect performance resulted in reduced plant health, slow insect growth, and increased necrosis at feeding sites. This reduced the reliability of the trial and samples viable for downstream DNA or RNA sequencing. However, leaf performance analyses remained robust. Because sexual reproduction requires the leaf feeding form (and is thus the biggest driver of adaptation in the field) we modified our performance analysis to include leaf-level performance. We also added two additional rootstocks of similar parentage to allow for collection of the same number of samples given two genotypes originally planned for use are immune.

Data:
Fig 1. Colonization success of our phylloxera clone varies across rootstocks (left). Although some evidence of gall induction is present on 3309C, thus far all insects died prior to reproduction. SW was used to replace 3309C and 110R was replaced with 99R of similar parentage due to lack of available plants at the start of the experiment. Nonetheless, a gradient of performance success has been established and trials are ongoing (right panel).



2. Identify genotyping targets of functional importance to improve field assessments of phylloxera diversity.

Summary: Given variation in colonization success (including insect failure/rootstock success) we have established a gradient to maximize the effects of host adaption. We have collected generation zero (the colonizing generation) samples and submitted these for sequencing to verify heritable change from adult to egg. With the trial (Obj 1) ongoing, we anticipate all remaining samples will be collected and sequenced in Spring 2022.

3. Validate this predictive framework on historic samples.

Summary: We have attempted contact numerous times with A. Walker (via phone and email) to retrieve his samples but have received no response. We have since collected other samples of known host and location from other colleagues and state agencies. We have successfully optimized a DNA extraction protocol with very low input. Sample submission and sequencing is expected during Spring 2022.

Modifications: We originally planned to sequence samples from a nationwide collecting trip (see Lund et al. 2017); however, these samples may no longer be available. Other samples from growing regions in WA, CA, and the Midwest/eastern US are being amassed from different collection times (including historic samples) and of known host and location.

4. Disseminate research results through open access venues.

As data become available we will host summaries and photos documenting progress on the project webpage (http://www.nabitylab.org/grape-phylloxera.html). Research results will be submitted for publication after completion of the project in 2022.